Claims

1. A nucleic acid molecule comprising (i) a first nucleotide sequence encoding a recombinant T cell receptor (TCR) or an antigen binding portion thereof that specifically binds human gp100 ("anti-gp100 TCR"); and (ii) a second nucleotide sequence, wherein the second nucleotide sequence or the polypeptide encoded by the second nucleotide sequence inhibits the expression of an endogenous TCR,
wherein the anti-gp100 TCR cross competes for binding to human gp100 with a reference TCR, which comprises an alpha chain and a beta chain, and wherein the alpha chain comprises an amino acid sequence as set forth in SEQ ID NO: 1 and the beta chain comprises an amino acid sequence as set forth in SEQ ID NO: 2.

2. A nucleic acid molecule comprising (i) a first nucleotide sequence encoding a recombinant T cell receptor (TCR) or an antigen binding portion thereof that specifically binds human gp100 ("anti-gp100 TCR"); and (ii) a second nucleotide sequence, wherein the second nucleotide sequence or the polypeptide encoded by the second nucleotide sequence inhibits the expression of an endogenous TCR,
wherein the anti-gp100 TCR binds the same epitope or an overlapping epitope of human gp100 as a reference TCR, which comprises an alpha chain and a beta chain, wherein the alpha chain comprises an amino acid sequence as set forth in SEQ ID NO: 1 and the beta chain comprises an amino acid sequence as set forth in SEQ ID NO: 2.

3. The nucleic acid molecule of claim 1 or 2, wherein the anti-gp100 TCR binds to an epitope of gp100 consisting of an amino acid sequence as set forth in SEQ ID NO: 13.

4. The nucleic acid molecule of claim 2 or 3, wherein the epitope is complexed with an HLA class I molecule.

5. The nucleic acid molecule of claim 4, wherein the HLA class I molecule is an HLA-A, HLA-B, HLA-C, HLA-E, HLA-F, or HLA-G allele.

6. The nucleic acid molecule of claim 4, wherein the HLA class I molecule is an HLA-C*06 allele.

7. The nucleic acid molecule of any one of claims 4 to 6, wherein the HLA class I molecule is selected from an HLA-C*06:015 allele, an HLA-C*06:02 allele, an HLA-C*06:03 allele, an HLA-C*06:04 allele, an HLA-C*06:05 allele, an HLA-C*06:06 allele, an HLA-C*06:07 allele, and an HLA-C*06:086 allele.

8. The nucleic acid molecule of any one of claims 4 to 7, wherein the HLA class I molecule is an HLA-C*06:02 allele.

9. The nucleic acid molecule of any one of claims 1 to 8, wherein the anti-gp100 TCR comprises an alpha chain and a beta chain,
wherein the alpha chain comprises a variable region comprising an alpha chain CDR1, an alpha chain CDR2, and an alpha chain CDR3; and
wherein the beta chain comprises variable domain comprising a beta chain CDR1, a beta chain CDR2, and a beta chain CDR3;
wherein the alpha chain CDR3 comprises an amino acid sequence as set forth in SEQ ID NO: 7.

10. The nucleic acid molecule of claim 9, wherein the beta chain CDR3 of the anti-gp100 TCR comprises an amino acid sequence as set forth in SEQ ID NO: 10.

11. The nucleic acid molecule of any one of claims 1 to 8, wherein the anti-gp100 TCR comprises an alpha chain and a beta chain, wherein the alpha chain comprises a variable region comprising an alpha chain CDR1, an alpha chain CDR2, and an alpha chain CDR3; and
wherein the beta chain comprises variable domain comprising a beta chain CDR1, a beta chain CDR2, and a beta chain CDR3;
wherein the beta chain CDR3 of the anti-gp100 TCR comprises an amino acid sequence as set forth in SEQ ID NO: 10.

12. The nucleic acid molecule of claim 11, wherein the alpha chain CDR3 of the anti-gp100 TCR comprises an amino acid sequence as set forth in SEQ ID NO: 7.

13. The nucleic acid molecule of any one of claims 9 to 12, wherein the alpha chain CDR1 of the anti-gp100 TCR comprises an amino acid sequence as set forth in SEQ

ID NO: 5.

14. The nucleic acid molecule of any one of claims 9 to 13, wherein the beta chain CDR1 of the anti-gp100 TCR comprises an amino acid sequence as set forth in SEQ ID NO: 8.

15. The nucleic acid molecule of any one of claims 9 to 14, wherein the alpha chain CDR2 of the anti-gp100 TCR comprises an amino acid sequence as set forth in SEQ ID NO: 6.

16. The nucleic acid molecule of any one of claims 9 to 15, wherein the beta chain CDR2 of the anti-gp100 TCR comprises an amino acid sequence as set forth in SEQ ID NO: 9.

17. The nucleic acid molecule of any one of claims 9 to 16, wherein the alpha chain variable domain of the anti-gp100 TCR comprises an amino acid sequence of a variable domain present in the amino acid sequence set forth SEQ ID NO: 1.

18. The nucleic acid molecule of any one of claims 9 to 17, wherein the beta chain variable domain of the anti-gp100 TCR comprises an amino acid sequence of a variable domain present in the amino acid sequence set forth SEQ ID NO: 2.

19. The nucleic acid molecule of any one of claims 9 to 18, wherein the alpha chain of the anti-gp100 TCR further comprises a constant region, wherein the constant region is different from endogenous constant region of the alpha chain.

20. The nucleic acid molecule of any one of claims 9 to 19, wherein the alpha chain of the anti-gp100 TCR further comprises a constant region, wherein the alpha chain constant region comprises an amino acid sequence having at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity to a constant region present in the amino acid sequence set forth SEQ ID NO: 1.

21. The nucleic acid molecule of claim 19 or 20, wherein the alpha chain constant region

comprises an amino acid sequence comprising at least 1, at least 2, at least 3, at least 4, or at least 5 amino acid substitutions relative to a constant region present in the amino acid sequence set forth SEQ ID NO: 1.

22. The nucleic acid molecule of any one of claims 9 to 21, wherein the beta chain of the anti-gp100 TCR further comprises a constant region, wherein the constant region is different from endogenous constant regions of the beta chain.

23. The nucleic acid molecule of any one of claims 9 to 22, wherein the beta chain of the anti-gp100 TCR further comprises a constant region, wherein the beta chain constant region comprises an amino acid sequence having at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity to a constant region present in the amino acid sequence set forth SEQ ID NO: 2.

24. The nucleic acid molecule of claim 22 or 23, wherein the beta chain constant region comprises an amino acid sequence comprising at least 1, at least 2, at least 3, at least 4, or at least 5 amino acid substitutions relative to a constant region present in the amino acid sequence set forth SEQ ID NO: 2.

25. The nucleic acid molecule of any one of claims 9 to 24, wherein the alpha chain of the anti-gp100 TCR comprises an amino acid sequence as set forth in SEQ ID NO: 1.

26. The nucleic acid molecule of any one of claims 9 to 25, wherein the beta chain of the anti-gp100 TCR comprises an amino acid sequence as set forth in SEQ ID NO: 2.

27. The nucleic acid molecule of any one of claims 1 to 26, wherein the second nucleotide sequence is one or more siRNAs that reduce the expression of endogenous TCRs.

28. The nucleic acid molecule of claim 27, wherein the one or more siRNAs are complementary to a target sequence within a nucleotide sequence encoding a constant region of the endogenous TCRs.

29. The nucleic acid molecule of claim 27 or 28, wherein the one or more siRNAs

comprise one or more nucleotide sequences selected from the group consisting of SEQ ID NOs: 53-56.

30. The nucleic acid molecule of any one of claims 1 to 29, wherein the second nucleotide sequence encodes Cas9.

31. The nucleic acid molecule of any one of claims 1 to 30, wherein the anti-gp100 TCR comprises an alpha chain constant region, a beta chain constant region, or both; and wherein the alpha chain constant region, the beta chain constant region, or both comprises an amino acid sequence having at least 1, at least 2, at least 3, at least 4, or at least 5 substitutions within the target sequence relative to the corresponding amino acid sequence of an endogenous TCR.

32. A vector comprising the nucleic acid molecule of any one of claims 1 to 31.

33. The vector of claim 32, which is a viral vector, a mammalian vector, or bacterial vector.

34. The vector of claim 32 or 33, which is a retroviral vector.

35. The vector of any one of claims 32 to 34, which is selected from the group consisting of an adenoviral vector, a lentivirus, a Sendai virus vector, a baculoviral vector, an Epstein Barr viral vector, a papovaviral vector, a vaccinia viral vector, a herpes simplex viral vector, a hybrid vector, and an adeno associated virus (AAV) vector.

36. The vector of any one of claims 32 to 35, which is a lentivirus.

37. A T cell receptor (TCR) or an antigen binding portion thereof comprising the alpha chain variable domain of the anti-gp100 TCR of any one of claims 9 to 31 and the beta chain variable domain of the anti-gp100 TCR of any one of claims 9 to 31.

38. A recombinant T cell receptor (TCR) or an antigen binding portion thereof that specifically binds human gp100 ("an anti-gp100 TCR"), which cross competes for binding to human gp100 with a reference TCR;

wherein the reference TCR comprises an alpha chain and a beta chain, and wherein the alpha chain comprises an amino acid sequence as set forth in SEQ ID NO: 1 and the beta chain comprises an amino acid sequence as set forth in SEQ ID NO: 2; and

wherein the anti-gp100 TCR comprises an alpha chain and a beta chain, wherein the alpha chain comprises a constant region, and wherein the beta chain comprises a constant region; wherein

(i) the alpha chain constant region comprises an amino acid sequence having a least 1, at least 2, at least 3, at least 4, or at least 5 amino acid substitutions relative to a constant region present in the amino acid sequence set forth in SEQ ID NO: 1 or

(ii) the beta chain constant region comprises an amino acid sequence having a least 1, at least 2, at least 3, at least 4, or at least 5 amino acid substitutions relative to a constant region present in the amino acid sequence of SEQ ID NO: 2.

39. A recombinant T cell receptor (TCR) or an antigen binding portion thereof that specifically binds human gp100 ("an anti-gp100 TCR"), which binds the same epitope or an overlapping epitope of human gp100 as a reference TCR;

wherein the reference TCR comprises an alpha chain and a beta chain, and wherein the alpha chain comprises an amino acid sequence as set forth in SEQ ID NO: 1 and the beta chain comprises an amino acid sequence as set forth in SEQ ID NO: 2; an

wherein the anti-gp100 TCR comprises an alpha chain and a beta chain, wherein the alpha chain comprises a constant region, and wherein the beta chain comprises a constant region; wherein

(i) the alpha chain constant region comprises an amino acid sequence having a least 1, at least 2, at least 3, at least 4, or at least 5 amino acid substitutions relative to a constant region present in the amino acid sequence set forth in SEQ ID NO: 1 or

(ii) the beta chain constant region comprises an amino acid sequence having a least 1, at least 2, at least 3, at least 4, or at least 5 amino acid substitutions relative to a constant region present in the amino acid sequence set forth in SEQ ID NO: 2.

40. The anti-gp100 TCR of claim 38 or 39, which binds to an epitope of gp100 consisting of an amino acid sequence as set forth in SEQ ID NO: 13.

41. The anti-gp100 TCR of claim 39 or 40, wherein the epitope is complexed with an HLA class I molecule.

42. The anti-gp100 TCR of claim 41, wherein the HLA class I molecule is an HLA-A, HLA-B, HLA-C, HLA-E, HLA-F, or HLA-G allele.

43. The anti-gp100 TCR of claim 41 or 42, wherein the HLA class I molecule is an HLA-C*06 allele.

44. The anti-gp100 TCR of any one of claims 41 to 43, wherein the HLA class I molecule is selected from an HLA-C*06:01 allele, an HLA-C*06:02 allele, an HLA-C*06:03 allele, an HLA-C*06:04 allele, an HLA-C*06:05 allele, an HLA-C*06:06 allele, an HLA-C*06:07 allele, and an HLA-C*06:08 allele.

45. The anti-gp100 TCR of any one of claims 41 to 44, wherein the HLA class I molecule is an HLA-C*06:02 allele.

46. The anti-gp100 TCR of any one of claims 38 to 45, wherein the alpha chain of the anti-gp100 TCR comprises a variable domain comprising an alpha chain CDR1, an alpha chain CDR2, and an alpha chain CDR3; and
wherein the beta chain of the anti-gp100 TCR comprises variable domain comprising a beta chain CDR1, a beta chain CDR2, and a beta chain CDR3;
wherein the alpha chain CDR3 of the anti-gp100 comprises an amino acid sequence as set forth in SEQ ID NO: 7.

47. The anti-gp100 TCR of claim 46, wherein the beta chain CDR3 of the anti-gp100 TCR comprises an amino acid sequence as set forth in SEQ ID NO: 10.

48. The anti-gp100 TCR of any one of claims 38 to 45, wherein the alpha chain of the anti-gp100 TCR comprises a variable domain comprising an alpha chainCDR1, an alpha chain CDR2, and an alpha chain CDR3; and
wherein the beta chain of the anti-gp100 TCR comprises a variable domain comprising a beta chain CDR1, a beta chain CDR2, and a beta chain CDR3;

wherein the beta chain CDR3 of the anti-gp100 TCR comprises an amino acid sequence as set forth in SEQ ID NO: 10.

49. The anti-gp100 TCR of claim 48, wherein the alpha chain CDR3 of the anti-gp100 TCR comprises an amino acid sequence as set forth in SEQ ID NO: 7.

50. The anti-gp100 TCR of claim 49, wherein the alpha chain CDR1 of the anti-gp100 TCR comprises an amino acid sequence as set forth in SEQ ID NO: 5.

51. The anti-gp100 TCR of any one of claims 46 to 50, wherein the beta chain CDR1 of the anti-gp100 TCR comprises an amino acid sequence as set forth in SEQ ID NO: 8.

52. The anti-gp100 TCR of any one of claims 46 to 51, wherein the alpha chain CDR2 of the anti-gp100 TCR comprises an amino acid sequence as set forth in SEQ ID NO: 6.

53. The anti-gp100 TCR of any one of claims 46 to 52, wherein the beta chain CDR2 of the anti-gp100 TCR comprises an amino acid sequence as set forth in SEQ ID NO: 9.

54. The anti-gp100 TCR of any one of claims 46 to 53, wherein the alpha chain variable domain of the anti-gp100 TCR comprises an amino acid sequence of a variable domain present in the amino acid sequence set forth in SEQ ID NO: 1.

55. The anti-gp100 TCR of any one of claims 46 to 54, wherein the beta chain variable domain of the anti-gp100 TCR comprises an amino acid sequence of a variable domain present in the amino acid sequence set forth in SEQ ID NO: 2.

56. The anti-gp100 TCR of any one of claims 38 to 55, wherein the alpha chain constant region comprises an amino acid sequence having at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity to the amino acid sequence of a constant region present in the amino acid sequence set forth in SEQ ID NO: 1.

57. The anti-gp100 TCR of any one of claims 38 to 56, wherein the beta chain constant

region comprises an amino acid sequence having at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity to the amino acid sequence of a constant region present in the amino acid sequence set forth in SEQ ID NO: 2.

58. The anti-gp100 TCR of any one of claims 38 to 57, wherein the alpha chain of the anti-gp100 TCR comprises an amino acid sequence as set forth in SEQ ID NO: 1.

59. The anti-gp100 TCR of any one of claims 38 to 58, wherein the beta chain of the anti-gp100 TCR comprises an amino acid sequence as set forth in SEQ ID NO: 2.

60. A bispecific TCR comprising a first antigen-binding domain and a second antigen-binding domain, wherein the first antigen-binding domain comprises the TCR or an antigen-binding portion thereof of claim 37 or the TCR or an antigen-binding portion thereof of any one of claims 38 to 59.

61. The bispecific TCR of claim 60, wherein the first antigen-binding domain comprises a single chain variable fragment ("scFv").

62. The bispecific TCR of claim 60 or 61, wherein the second antigen-binding domain binds specifically to a protein expressed on the surface of a T cell.

63. The bispecific TCR of any one of claims 60 to 62, wherein the second antigen-binding domain binds specifically to CD3.

64. The bispecific TCR of any one of claims 60 to 63, wherein the second antigen-binding domain comprises an scFv.

65. The bispecific TCR of any one of claims 60 to 64, wherein the first antigen-binding domain and the second antigen-binding domain are linked or associated by a covalent bond.

66. The bispecific TCR of any one of claims 60 to 65, wherein the first antigen-binding domain and the second antigen-binding domain are linked by a peptide bond.

67. A cell comprising the nucleic acid molecule of any one of claims 1 to 31, the vector of any one of claims 32 to 36, the TCR of claim 37, the recombinant TCR of any one of claims 38 to 59, or the bispecific TCR of any one of claims 60 to 66.

68. The cell of claim 67, which further expresses CD3.

69. The cell of claim 67 or 68, which is selected from the group consisting of a T cell, a natural killer (NK) cell, an natural killer T (NKT) cell, or an ILC cell.

70. Use of the cell of any one of claims 67 to 69 in the manufacture of a medicament for treating a cancer in a subject in need thereof.

71. The use of claim 70, wherein the cancer is selected from the group consisting of melanoma, bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, uterine cancer, ovarian cancer, rectal cancer, cancer of the anal region, stomach cancer, testicular cancer, uterine cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, Hodgkin's Disease, non-Hodgkin's lymphoma (NHL), primary mediastinal large B cell lymphoma (PMBC), diffuse large B cell lymphoma (DLBCL), follicular lymphoma (FL), transformed follicular lymphoma, splenic marginal zone lymphoma (SMZL), cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis, chronic or acute leukemia, acute myeloid leukemia, chronic myeloid leukemia, acute lymphoblastic leukemia (ALL) (including non T cell ALL), chronic lymphocytic leukemia (CLL), solid tumors of childhood, lymphocytic lymphoma, cancer of the bladder, cancer of the kidney or ureter, carcinoma of the renal pelvis, neoplasm of the central nervous system (CNS), primary CNS lymphoma, tumor angiogenesis, spinal axis tumor, brain stem glioma, pituitary adenoma, Kaposi's sarcoma, epidermoid cancer, squamous cell cancer, T-cell lymphoma, environmentally induced cancers including those induced by asbestos, other B cell malignancies, and combinations of said cancers.

72. The use of claim 70 or 71, wherein the cancer is relapsed or refractory.

73. The use of any one of claims 70 to 72, wherein the cancer is locally advanced.

74. The use of any one of claims 70 to 73, wherein the cancer is advanced.

75. The use of any one of claims 70 to 74, wherein the cancer is metastatic.

76. The use of any one of claims 70 to 75, wherein the cells are obtained from the subject.

77. The use of any one of claims 70 to 76, wherein the cells are obtained from a donor other than the subject.

78. The use of any one of claims 70 to 77, wherein the subject is preconditioned prior to the administering of the cells.

79. The use of any one of claims 68 to 78, wherein the preconditioning comprises a chemotherapy, a cytokine, a protein, a small molecule, or any combination thereof which is to be be administered to the subject.

80. The use of claim 78 or 79, wherein the preconditioning comprises an interleukin, which is to be administered to the subject.

81. The use of any one of claims 78 to 80, wherein the preconditioning comprises IL-2, IL-4, IL-7, IL-9, IL-15, IL-21, or any combination thereof, which is to be administered to the subject.

82. The use of any one of claims 78 to 81, wherein the preconditioning comprises a preconditioning agent selected from the group consisting of cyclophosphamide, fludarabine, vitamin C, an AKT inhibitor, ATRA, Rapamycin, or any combination thereof, which is to be administered to the subject.

83. The use of any one of claims 78 to 82, wherein the preconditioning comprises cyclophosphamide, fludarabine, or both, which is to be administered to the subject.

84. A method of engineering an antigen-targeting cell, comprising transducing a cell collected from a subject in need of a T cell therapy with the nucleic acid molecule of any one of claims 1 to 31 or the vector of any one of claims 32 to 36.

85. The method of claim 84, wherein the antigen-targeting cell further expresses CD3.

86. The method of claim 84 or 85, wherein the cell is a T cell or a natural killer (NK) cell.

87. An HLA class I molecule complexed to a peptide, wherein the HLA class I molecule comprises an α1 domain, an α2 domain, an α3 domain and a β2m, and wherein the peptide consists of an amino acid sequence as set forth in SEQ ID NO: 14.

88. The HLA class I molecule of claim 87, which is an HLA-A, HLA-B, HLA-C, HLA-E, HLA-F, or HLA-G.

89. The HLA class I molecule of claim 87 or 88, which is an HLA-C.

90. The HLA class I molecule of any one of claims 87 to 89, which is an HLA-C*06 allele.

91. The HLA class I molecule of any one of claims 87 to 90, wherein the HLA class I molecule is selected from an HLA-C*06:01 allele, HLA-C*06:02 allele, an HLA-C*06:03 allele, an HLA-C*06:04 allele, an HLA-C*06:05 allele, and an HLA-C*06:06 allele.

92. The HLA class I molecule of any one of claims 87 to 91, wherein the HLA class I molecule is an HLA-C*06:02 allele.

93. The HLA class I molecule of any one of claims 87 to 92, wherein the HLA class I molecule is an HLA-C*03:03 allele.

94. The HLA class I molecule of any one of claims 87 to 93, which is a monomer.

95. The HLA class I molecule of any one of claims 87 to 93, which is a dimer.

96. The HLA class I molecule of any one of claims 87 to 93, which is a trimer.

97. The HLA class I molecule of any one of claims 87 to 93, which is a tetramer.

98. The HLA class I molecule of any one of claims 87 to 93, which is a pentamer.

99. An antigen presenting cell (APC), comprising the HLA class I molecule of any one of claims 87 to 98.

100. The APC of claims 99, wherein the HLA class I molecule is expressed on the surface of the APC.

101. A method of enriching a target population of T cells obtained from a human subject, comprising contacting the T cells with the HLA class I molecule of any one of claims 87 to 98 or the APC of claim 99 or 100, wherein following the contacting, the enriched population of T cells comprises a higher number of T cells capable of binding the HLA class I molecule relative to the number of T cells capable of binding the HLA class I molecule prior to the contacting.

102. A method of enriching a target population of T cells obtained from a human subject, comprising contacting the T cells *in vitro* with a peptide, wherein the peptide consists of an amino acid sequence as set forth in SEQ ID NO: 13, wherein following the contacting, the enriched population of T cells comprises a higher number of T cells capable of targeting a tumor cell relative to the number of T cells capable of targeting a tumor cell prior to the contacting.

103. The method of claim 101 or 102, wherein the T cells obtained from the human subject are tumor infiltrating lymphocytes (TIL).

104. Use of the enriched T cells of claim 101 or 102 in the manufacture of a medicament for treating a tumor in a subject in need thereof

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105. Use of a peptide having an amino acid sequence as set forth in SEQ ID NO: 13 in the manufacture of a medicament for enhancing cytotoxic T cell-mediated targeting of cancer cells in a subject afflicted with a cancer.

106. A cancer vaccine comprising a peptide having an amino acid sequence as set forth in SEQ ID NO: 13.

107. A method of selecting a T cell capable of targeting a tumor cell, comprising contacting a population of isolated T cells *in vitro* with a peptide, wherein the peptide consists of an amino acid sequence as set forth in SEQ ID NO: 11.

108. The method of claim 107, wherein the T cell is a tumor infiltrating lymphocytes (TIL).

109. The cell of any one of claims 67 to 69 for use in treating a cancer in a subject in need thereof